Effects of chloroquine on male fertility in Wistar rats

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This study reports the effects of chloroquine on testicular function in Wistar rats. Weekly administration of the drug (40 or 50 mg/kg body weight) reduced epididymal sperm number without affecting the proportions of motile or abnormal sperm. There was a significant reduction in testicular DNA content with both doses tested. Although a slight reduction in testicular weight was recorded in the 50-mg group only, the number of testicular germ cells was reduced with both doses tested. There were no significant changes in the weights of the accessory organs and in the levels of circulating androgens and gonadotrophins. However, chloroquine reduced litter size and increased the incidence of stillbirths and abnormal pups.

The drug appears to be deleterious both at the testicular and epididymal levels, although the latter could be the result of the former. Whereas the use of chloroquine in recommended therapeutic doses for malaria is unlikely to cause serious fertility problems, exploitation of the anti-inflammatory properties of higher doses of the drug requires further consideration.

Dié verslag handel oor die uitwerking van chlorokien op die testikelfunksie by Wistar-rotte. 'n Weeklikse toediening van die middel (40 of 50 mg/kg liggaamsmassa) het die getal epididimissperme verminder sonder dat die persentasie beweeglike of abnormale sperme geraak is. Daar was by albei doseerpeile 'n beduidende afname in testikel-DNA. Hoewel die testikelmassa slegs by die groep met 'n dosering van 50 mg laer was as by die ander, het die getal testikelkiemselle by albei doseringsgroepe afgeneem. Daar is geen noemenswaardige massaverskille waargeneem by enige neweorgane nie en ook nie in die vlak van sirkulerende androgene en gonadotropiene nie. Chlorokien het egter veroorsaak dat die werpsels minder talryk was en dat daar meer doodgebore en abnormale kleintjies was.

Dit lyk asof die middel 'n nadelige uitwerking op testikelsowel as epididimisvlak het, hoewel die laasgenoemde 'n gevolg van die eersgenoemde kan wees. Terwyl dit onwaarskynlik is dat chlorokien teen die terapeutiese dosisse wat vir malaria aanbeveel word, enige ernstige probleme met vrugbaarheid sal oplewer, behoort verdere aandag aan die middel se antiinflammatoriese werking by hoër dosisse geskenk te word.

Chloroquine is widely used for the suppression and treatment of malaria. Although this drug was primarily developed as an antimalarial agent, its anti-inflammatory properties are exploited in the treatment of rheumatoid arthritis and discoid lupus erythematosus.[1] The drug is readily absorbed from the gastro-intestinal tract and distributed throughout the body.[2] It is excreted mainly via the urine, either as unchanged drug or as metabolites. Although the dosage for suppression of malaria is only 400 mg once weekly, long-term treatment of disease other than malaria may involve chloroquine administration of 250 to 750 mg daily for months or even years.[1]

Although the mechanism of plasmodicidal action of chloroquine is not fully understood, it is thought to act through DNA binding.[1] It binds with DNA in the double-stranded form only and inhibits both DNA and RNA polymerase, the former more so than the latter.[3] It is also suggested that chloroquine may inhibit protein metabolism by blocking the sulphydryl – disulphide interchange reaction.[4]

Relatively few toxicity problems are associated with the use of chloroquine for malaria and prophylaxis. However, when the anti-inflammatory properties of high doses of the drug are exploited in the treatment of rheumatoid arthritis and lupus erythematosus, the incidence of side effects is higher, the most serious problem being irreversible retinopathy.[4] There have also been reports of foetal damage as a result of chloroquine therapy during pregnancy.[1,4]

Apart from reports that antimalarial drugs may reduce sperm number[5] and produce oestrogen-secreting tumours in the testis,[6] the only extensive investigation (as far as the authors are aware) of the effects of chloroquine on male fertility has been undertaken by Darod.[7] He has shown that 50 mg chloroquine/kg per week administered to rats maintained normal growth rate and produced plasma drug concentrations comparable to human therapeutic levels. Although libido was not affected by treatment, fertility tests indicated a reduction in pregnancy rate and litter size. In addition, by surgically separating the epididymis from the testis, Darod demonstrated that the probable point of chloroquine action is at the level of the epididymis. In such surgically manipulated animals there was a reduction in sperm number, an increase in the proportion of abnormal sperm, a decrease in progressive sperm motility and in the survival time of epididymal sperm. Concomitant treatment with chloroquine and testosterone produced normal epididymal sperm, suggesting that chloroquine lowers androgen levels. There is also evidence to suggest that chloroquine causes accumulation of epidermal growth factor in pituitary and granulosa cells, and that the growth factor inhibits testicular steroidogenesis *in vitro*.[8,9] It is possible that chloroquine could inhibit testosterone synthesis *in vivo* by affecting some intratesticular factor in a similar manner.

The present investigations with chloroquine were therefore undertaken to confirm that chloroquine impaired male fertility, as has been suggested previously by isolated reports. Secondly, it seemed necessary to ascertain whether androgen and gonadotrophin levels are altered by treatment, as has been suggested by indirect evidence,[7-9] and finally to determine the probable site of antifertility action of the drug.

Materials and methods

Experimental design. Experiments were conducted on sexually mature male Wistar rats (~ 250 g). Food and water were supplied ad libitum. Injectable chloroquine sulphate (Nivaquine, Maybaker) was administered to experimental animals intraperitoneally in doses of 40 or 50 mg/kg per week for 12 weeks. Control animals received an equal volume of physiological saline.

Fertility tests. Treated and control males were placed individually with experienced females during the last 5 days of treatment. The female rats were examined for vaginal plugs twice daily, and were subsequently observed for at least 3 weeks to detect pregnancy. Pups were counted and visually examined for abnormalities.

Tissue sampling. Animals were sacrificed by decapitation under ether anaesthesia between 08h00 and 12h00. Blood was collected from the neck, plasma was separated and stored at $-20°C$ for hormone assay. Sperm were extracted from the right cauda epididymis to assess number, motility and morphology.[10] The left testis was used for biochemical analysis. The left epididymis was fixed in Bouin-Hollande fixative, routinely processed for microtomy, sectioned at 5 μm and stained with Haematoxylin and Eosin. Histological measurements were made under the ×40 objective on a light microscope fitted with a stage-ocular micrometer combination.

Extraction and measurement of DNA, RNA and protein. DNA, RNA and protein were extracted by a modification of the Schmidt–Thannhauser procedure.[11] The colour reaction between diphenylamine and DNA[12] formed the basis for the measurement of DNA. Protein was measured with the Folin phenol reagent after alkaline copper treatment,[13] and RNA was measured after treatment with perchloric acid.

Determination of testicular cell number and size. The number and size of cells in the testis were determined according to the method of Enesco and Leblond,[14] which makes use of the following formulae:

$$\text{No. of cells in millions} = \frac{\text{mg testicular DNA} \times 10^3}{6{,}2}$$

6,2 being the DNA content (pg) of a diploid nucleus.

$$\text{Cell size (ng)} = \frac{\text{testicular weight (g)} \times 10^3}{\text{no. of cells in millions}}.$$

Radioimmunoassay for plasma androgens and gonadotrophins. Plasma testosterone levels were determined with the Immuchem covalent coat direct ^{125}I testosterone assay kit (Immuchem Corp., USA), which detects the total unconjugated form of the steroid. Details of the assay are described in the product information booklet. Both the inter-assay and intra-assay coefficient of variation were less than 10%.

Plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were measured by standard double antibody radioimmunoassays[15] using rat immuno-reagents supplied by the National Hormone and Pituitary Program (USA). Hormone concentrations are expressed in terms of the NIADDK rat LH reference preparation (rLH-RP-2) and the NIADDK rat FSH reference preparation (rFSH-RP-2). The intra-assay coefficient of variation for LH was 6,8% and that for FSH 6,5%. The inter-assay coefficient of variation for both assays was less than 10%.

Statistical analysis. Experimental data were subjected to an analysis of variance. If an overall significance was noted, individual treatment means were separated by the least significance difference test.

Results

Chloroquine treatment reduced epididymal sperm number, testicular weight and testicular DNA content (Table 1). The epididymal sperm number was reduced by 27% in the 40-mg group and by 30% in the 50-mg group ($P<0{,}025$). There was a 12% reduction in testicular weight in the 50-mg group only ($P<0{,}02$). The number of testicular cells decreased by 24% in the 40-mg group ($P<0{,}05$) and by 36% in the 50-mg group ($P<0{,}01$; Table 2). There was no significant change in the size of testicular cells in treated rats. The DNA content in the testis dropped by 18% in the 40-mg group and by 27% in the 50-mg group ($P<0{,}05$). Chloroquine treatment did not alter the proportion of motile or abnormal sperm in the cauda epididymis, nor was there a change in weight of the epididymis or seminal vesicle (Table 3). Testicular RNA and protein contents ~~were~~ also unaffected by treatment (Table 4). Histological examination of the epididymis did not indicate any difference between control and treated animals in the height of the epithelial layer or in the proportion of duct transverse sections devoid of sperm (Table 5). Neither did plasma testosterone decrease or gonadotrophin levels increase significantly in the treated rats (Table 4). However, a statistically non-significant rise in FSH was recorded in treated rats. Chloroquine also increased the number of stillbirths and decreased the total number of pups sired (Table 6).

Discussion

Our results indicate that chloroquine has deleterious effects on male sexual function. The most pronounced effects of the drug are those on testicular germ cells and epididymal sperm. The oligozoospermia could be due to the reduction in testicular germ cells. Alternatively, the drug could be acting at both the testicular and epididymal levels. However, the possible inhibition by chloroquine of some factor(s) involved in intratesticular control[16] cannot be ruled out. Support for this is provided by reports that the drug inhibits the mitogenic activity of epidermal growth factor.[8] Chloroquine could similarly inhibit the mitogenic factor thought to be produced by the testis.[17]

Fertility tests in our experiments showed that treated rats generally produced smaller litters, with a significant proportion of stillbirths and abnormal pups. Darod[7] has also reported a reduction in pregnancy rate and litter size. Although reduced litters could be due partly to the germ cell damage and the oligozoospermia, it is also possible, as seen with some chemical agents in animal studies,[18] that chloroquine alters the fertilizing capacity of sperm without any other obvious effects on the ejaculate.

Table 1. Some adverse effects of chloroquine treatment† in rats (mean ± s.e.m.)

Group	Sperm no. (×10^6/cauda)	Testis weight (mg)	Testicular DNA (μg/g testis)
Control	117 ± 5,8	1520 ± 57	111 ± 3,3
40 mg	85,5 ± 6,0*	1383 ± 55	91 ± 7,5*
50 mg	82,5 ± 11,0*	1338 ± 35*	81 ± 7,2*

n = 7. *Significantly different from controls. †Animals received 40 or 50 mg drug/kg per week for 12 weeks.

Table 2. Effect of chloroquine† on testicular cell number and size (mean ± s.e.m.)

Group	Testicular germ cells (×10^6)	Cell size (pg)
Control	27,3 ± 1,6	56 ± 2
40 mg	20,7 ± 2,4*	71 ± 7
50 mg	17,5 ± 1,7*	82 ± 11

n = 7. *Significantly different from controls. †Animals received 40 or 50 mg drug/kg per week for 12 weeks.

Table 3. Effect of chloroquine* on epididymal sperm and organ weights in rats (mean ± s.e.m.)

	Epididymal sperm		Epididymis	seminal vesicle
Group	abnormality (%)	motility (%)	weight (mg)	
Control	6 ± 0,8	80 ± 1,9	518 ± 19	704 ± 52
40 mg	5 ± 1,1	81 ± 2,6	503 ± 18	651 ± 31
50 mg	4 ± 0,8	84 ± 1,9	548 ± 48	700 ± 72

n = 7. *Animals received 40 or 50 mg drug/kg per week for 12 weeks.

Table 4. Effect of chloroquine* on hormone, nucleic acid and protein levels (mean ± s.e.m.)

Group	Testosterone (pg/ml)	LH	FSH	RNA	Protein
		(ng/ml)		µg/g testis	
Control	2255 ± 488	1,3 ± 0,3	12,3 ± 2,3	15 ± 1,9	151 ± 11
40 mg	2419 ± 597	1,3 ± 0,1	22,8 ± 3,8	15 ± 1,8	155 ± 11
50 mg	2075 ± 502	1,3 ± 0,2	18,5 ± 2,2	15 ± 4,0	183 ± 15

n = 7. *Animals received 40 or 50 mg drug/kg per week for 12 weeks.

Table 5. Histological assessment of epididymal epithelium height and epididymal sperm after chloroquine treatment (mean ± s.e.m.)

Group	Epithelial height (µm)	Duct cross-sections* devoid of sperm (%)
Control	18,2 ± 0,7	18,2 ± 1,1
40 mg	18,5 ± 0,2	18,5 ± 3,5
50 mg	19,1 ± 1,0	21,8 ± 3,4

n = 7. *For each animal 20 measurements were taken and 14 duct cross-sections scored.

Table 6. Fertility tests in rats treated with chloroquine*

Group	Total no. of pups sired	No. stillborn	No. abnormal
Control	40	0	0
40 mg	5	0	0
50 mg	19	4	3

n = 7. Each male was mated individually with a receptive female. *Animals received 40 or 50 mg drug/kg per week for 12 weeks.

The reduction in testicular DNA levels in treated animals conforms with reports that chloroquine inhibits nucleic acid synthesis.[2] However, no change was noted in testicular RNA. Although the exact reason for this is unclear at this stage, it is known that DNA polymerase is more sensitive to chloroquine than is RNA polymerase. This could explain the reduction in DNA but not in RNA.

The present study did not demonstrate a significant reduction in circulating androgen levels or increases in gonadotrophin levels. However, Darod[7] has shown that testosterone administered with chloroquine to rats with surgically isolated epididymides reversed the antifertility effects of the drug, thereby suggesting that androgen withdrawal occurs in chloroquine-treated rats. In such a case, one would expect an androgen deficiency in the epididymis because of its separation from the testis. It seems as though the effects of chloroquine on epididymal sperm and fertility may be more pronounced in the isolated epididymis; this is because there is no renewal of sperm from the testis and because testosterone (largely bound to ABP) in the rete testis fluid cannot reach the epididymis. This is more likely than Darod's suggestion of androgen deficiency in chloroquine-treated rats, since there was no evidence in our studies that the effects of chloroquine were mediated via the hypothalamo-pituitary-gonadal axis. Androgen and gonadotrophin levels were unchanged, accessory organ weights were not affected and epididymal histology was normal. It therefore seems that in rats with isolated epididymides, chloroquine appears more deleterious than in the intact rats simply because of the physical effects of surgery, i.e. separation of epididymis from testis and thus prevention of sperm renewal in the former.

The reduction in the number of testicular cells in treated rats correlates with a reduction in testicular weight and with a rise in circulating FSH (though not statistically significant). It is now well established that raised FSH levels are indicative of tubular damage, the increase in FSH being due to improper feedback because of altered inhibin secretion. Testicular germ cell counts are necessary to determine which cell(s) are affected by the drug. The importance of determining whether stem cell spermatogonia are affected is clear since damage to these cells would lead to sterility.

There also exists a possibility that chloroquine adversely affects intratesticular control. However, this is difficult to ascertain since our understanding of testicular paracrinology is still in its infancy.

The routine use of chloroquine in currently recommended therapeutic doses for the suppression of malaria is unlikely to cause serious antifertility problems. However, the exploitation of the anti-inflammatory properties of higher doses of the drug for other diseases may have serious implications, especially since the present results show that chloroquine reduces litter size and increases the incidence of stillbirths and abnormal pups.

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1. Rollo I.M. (1975). Drugs used in the chemotherapy of malaria. In *The Pharmacological Basis of Therapeutics*, edit. L.S. Goodman & A. Gilman, 5th edn. Macmillan, New York.
2. Barrett-Conor E. (1982). Drugs for the treatment of parasitic infection. *Med. Clin. N. Am.* **66**, 245.
3. Allison J.L., O'Brien R.C. and Hahn F.E. (1965). DNA: reaction with chloroquine. *Science* **149**, 1111 – 1113.
4. Tester-Dalderup (1980). Antiprotozoal drugs. In *Meyler's Side Effects of Drugs. An Encyclopaedia of Adverse Reactions and Interactions*, 9th edn. Excerpta Medica, Amsterdam.
5. Stewart D.H. (1975). Drugs that cause and cure male fertility. *Drug Therapy* **5**, 42 – 49.
6. Bercovici J.P. *et al.* (1982). Interstitial testicular oestrogen-secreting tumours and chloroquine. *Lancet* **ii**, Oct. 817.
7. Darod M.F. (1984). *Study of the possible role of antimalarial drugs on male fertility*. MSc thesis, University of Kartoum, Sudan.
8. King A.C. and Cuatrecases P. (1981). Peptide hormone induced receptor mobility, aggregation and internalization. *N. Engl. J. Med.* **305**, 77 – 88.
9. Hsueh A.J.W., Welsh T.H. and Jones P.B.C. (1981). Inhibition of ovarian and testicular steroidogenesis by epidermal growth factor. *Endocrinology* **108**, 2002 – 2004.
10. Vawda A.I. and Davies A.G. (1986). An investigation into the effects of ketoconazole on testicular function in Wistar rats. *Acta Endocrinologica* **111**, 246 – 251.
11. Munro H.N. and Fleck A. (1966). The determination of nucleic acids. In *Methods of Biochemical Analysis*, edit. D. Glick, vol. XIV. Interscience, New York.
12. Burton K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**, 315 – 322.
13. Lowry D.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265 – 275.
14. Enesco M. and Leblond C.P. (1962). Increase in cell number as a factor in the growth of the organs and tissues of the young male rat. *J. Embryol. exp. Morphol.* **10**, 530 – 562.
15. Naik S.I., Young L.S., Charlton H.M. and Clayton R.N. (1984). Pituitary gonadotrophin-releasing hormone regulation in mice. 1 : Males. *Endocrinology* **115**, 106 – 113.
16. Sharpe R.M. (1984). Intratesticular factors controlling testicular function. *Biol. Reprod.* **30**, 29 – 49.
17. Feig L.A., Bellve A.R., Erickson N.H. and Klagsbrun M. (1980). Sertoli cells contain a mitogenic polypeptide. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4774 – 4778.
18. Mann *et al.* Reproductive function of males. In *Methods for Assessing the Effects of Chemicals on Reproductive Functions*. Scope 20, edit. V.B. Vouk and P.J. Sheehan. Wiley, New York.